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REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	28 December 1996	Final

4. TITLE AND SUBTITLE
"Enhanced TCE Degradation Using Genetically-Engineered MIcroorganisms"

5. FUNDING NUMBERS
DAAL03-92-G-0398

6. AUTHOR(S)
Thomas K. Wood

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)
University of California, Irvine
Irvine, CA 92697-2575

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
ARO 30871.7-LS-YIP

11. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
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12 b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Novel recombinant TCE-degrading bacteria were created using the best known enzyme for TCE degradation, soluble methane monooxygenase(sMMO) of the soil bacterium *Methylosinus trichosporium* OB3b. sMMO degrades a wide range of halogenated hydrocarbons (HCFCs, chloroform, dichloroethane, etc.), and it degrades TCE 100 times faster than any other microbial enzyme. The *mmo* genes were cloned and expressed in *Pseudomonas putida* F1, *Agrobacterium tumefaciens*, and *Rhizobium meliloti* using plasmids pSMMO20 and pSMMO40 created by the Wood laboratory. In addition, a novel fixed-film bioreactor has been constructed and optimized to mineralize TCE in the gas phase.

14. SUBJECT TERMS	15. NUMBER IF PAGES
TCE degradation, recombinant microorganisms, biofilter	3
	16. PRICE CODE

17. SECURITY CLASSIFICATION OR REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

ENHANCED TRICHLOROETHYLENE DEGRADATION USING GENETICALLY-ENGINEERED MICROORGANISMS

Final Progress Report

Thomas K. Wood

28 December 1996

U. S. Army Research Office

Contract DAAL03-92-0398 (30871-LS-YIP)

University of California, Irvine

Specific Aims. The goal of this project is to design a genetically-engineered microorganism capable of rapidly and efficiently degrading trichloroethylene (TCE). Since the enzyme soluble methane monooxygenase (sMMO) of *Methylosinus trichosporium* OB3b degrades a wide range of halogenated hydrocarbons (HCFCs, chloroform, dichloroethane, etc.), and it degrades TCE 100 times faster than any other microbial enzyme, the *smmo* genes have been cloned and expressed in *Pseudomonas putida* F1, *Agrobacterium tumefaciens*, and *Rhizobium meliloti* using plasmids pSMMO20 and pSMMO40 created by the Wood laboratory.. In addition, a novel fixed-film bioreactor has been constructed and is being optimized to mineralize TCE in the gas phase.

Results. This Army Young Investigator award was used to express whole, active sMMO for the first time in a foreign host by creating a recombinant, TCE-degrading strain that no longer requires methane to induce sMMO, is not subject to copper suppression of transcription, and degrades TCE without competitive inhibition. The recombinant mineralizes completely both TCE and chloroform, and it grows faster than the wild-type source of the sMMO enzyme (*M. trichosporium* OB3b). However, this construct degrades TCE slower than the wild-type source of the genes, *M. trichosporium* OB3b [V_{max} = 5 nmol TCE/(min mg protein) for the recombinant vs. 40-500 nmol TCE/(min mg protein)], and the activity of sMMO in the recombinant is inconsistent. By complementing cell-free extracts with purified sMMO, the inconsistent sMMO activity of the recombinant was determined to be due to incomplete activity of the hydroxylase component. Chemostat-grown cells were also used to determine that sMMO activity increases with growth rate and that the metabolic state of the cells affects enzyme consistency. Furthermore, it was also determined that the *in vitro* activity of sMMO is inhibited strongly by Cu(I), Cu(II), Ni(II), Zn(II), and chloramphenicol through their action on both the reductase and hydroxylase components.

In addition to studying sMMO, this grant was used to study the kinetics of other TCE-degrading strains (determining V_{max} and K_m for the first time for *B. cepacia* G4 PR1, *P. mendocina* KR1, and *P. putida* F1). To find a suitable bacterium for a fixed-film bioreactor, the extent of TCE degradation and mineralization was also compared on a common basis for five of the most common and active strains (*B. cepacia* G4, *B. cepacia* G4 PR1, *P. mendocina* KR1, *M. trichosporium* OB3b, and *P. putida* F1). To analyze the extent of mineralization of TCE in the fixed-bed bioreactor without disturbing the active biofilm, a minimal growth medium was formulated that lacks chloride ions so that chloride ions generated by TCE mineralization may be quantified with a chloride-ion specific electrode. An indole-containing, minimal-medium agar plate selection technique was also developed to indicate active expression of the TCE-degrading enzyme toluene-*ortho* monooxygenase as well as distinguish it from other TCE-degrading enzymes. A novel fixed-film bioreactor using *B. cepacia* G4 PR1 has also been designed and operated to degrade 242 μg TCE/L for up to 90 days.

Two graduate students have been trained on this grant: Deokjin Jahng received a Ph.D. degree in biochemical engineering (1995) and is now an assistant professor in the Department of Chemical Engineering at Myongji University (South Korea), and Adam Sun has received partial support and is currently a Ph.D. candidate at UC Irvine.

Publications:

1. "Electroporation of Pink-Pigmented Methylotrophic Bacteria," C. S. Kim and T. K. Wood, submitted to *Applied Biochemistry & Biotechnology* (1996).
2. "Trichloroethylene Mineralization in a Fixed-Film Bioreactor Using a Pure Culture Expressing Constitutively Toluene *ortho*-Monooxygenase, A. K. Sun and T. K. Wood, accepted by *Biotechnology and Bioengineering* (1996).
3. "Creating Auxotrophic Mutants in *Methylophilus methylotrophus* AS1 by Combining Electroporation and Chemical Mutagenesis," C. S. Kim and T. K. Wood, submitted to *Applied Microbiology & Biotechnology* (1996).
4. "Trichloroethylene Degradation and Mineralization by Pseudomonads and *Methylosinus trichosporium* OB3b," A. K. Sun and T. K. Wood, *Applied Microbiology & Biotechnology* **45**, 248-256 (1996).

5. "Optimization of Trichloroethylene Degradation Using Soluble Methane Monooxygenase of *Methylosinus trichosporium* OB3b Expressed in Recombinant Bacteria," D. Jahng, C. S. Kim, R. S. Hanson, and T. K. Wood, *Biotechnology and Bioengineering* **51**, 349-359 (1996).
6. "Metal Ions and Chloramphenicol Inhibition of Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b," D. Jahng and T. K. Wood, *Applied Microbiology & Biotechnology* **45**, 744-749 (1996).
7. "Trichloroethylene Degradation Using Recombinant Bacteria Expressing Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b," D. Jahng, A. K. Sun, C. S. Kim, and T. K. Wood, in *Molecular Biology of Pseudomonads*, p 280-288 (1996).
8. "Monitoring Trichloroethylene Mineralization by *Pseudomonas cepacia* G4 PR1", P. P. Luu, C. W. Yung, A. K. Sun, and T. K. Wood, *Applied Microbiology & Biotechnology* **44**, 259-264 (1995).
9. "Trichloroethylene and Chloroform Degradation by a Recombinant Pseudomonad Expressing Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b," D. Jahng and T. K. Wood, *Applied & Environmental Microbiology* **60**, 2473-2482 (1994).
10. "Expression of Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b in Recombinant Bacteria for Efficient Trichloroethylene Degradation," D. Jahng, Ph.D. Thesis, University of CA, Irvine (1995).

Presentations:

1. "TCE Mineralization in a Biofilter with a Pure Culture Which Constitutively Expresses Toluene *ortho*-Monooxygenase," A. K. Sun and T. K. Wood, American Society of Microbiology, New Orleans, LA National Meeting, May 21, 1996.
2. "Trichloroethylene Degradation Using Recombinant Bacteria Expressing Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b," *Pseudomonas* 1995, Tsukuba, Japan, August 24, 1995 (invited lecture).
3. "TCE Degradation Using Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b," Keystone Symposium on Environmental Biotechnology, Lake Tahoe, CA, March 20, 1995 (invited lecture).
4. "Trichloroethylene Degradation Using Recombinant Bacteria Expressing the Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b," Deokjin Jahng, Craig Kim, and T. K. Wood, American Chemical Society, Anaheim, CA National Meeting, April 4, 1995.
5. "Degradation and Mineralization of Gas-Phase Trichloroethylene Using a Fixed-Film Bioreactor," Adam K. Sun and T. K. Wood, American Institute of Chemical Engineers National Meeting, San Francisco, Nov. 18, 1994.
6. "TCE Degradation by a Recombinant Pseudomonad Containing the Soluble Methane Monooxygenase Gene of *M. trichosporium* OB3b," Deokjin Jahng and T. K. Wood, Emerging Technologies in Hazardous Waste Management VI, Atlanta, GA, September 19, 1994.
7. "TCE Degradation by a Recombinant Pseudomonad Containing the Soluble Methane Monooxygenase Gene of *M. trichosporium* OB3b," Deokjin Jahng and T. K. Wood, American Chemical Society, San Diego, CA National Meeting, March 15, 1994.

Personnel:

Deokjin Jahng (Ph.D. obtained July 1995)
Adam Sun (Ph.D. student)
Thomas K. Wood (Assistant Professor)